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Priority: 6-28-90 DE 4020607 Application filed on : 1-8-92 Patentblatt 92/07 Convention Countries named: AT BE CH DE DK ES FR GB GR IT LI LU NL SE	APPLICANT: BEHRINGWERKE Aktiengesellschaft P.O. Box 1140 W-3550 Marburg (DE) Applicant: THE GENERAL HOSPITAL CORPORATION Fruit Street (Bar-3) Boston, MA 02114 (US) Inventor: Dr. Leander Lauffer Walter-Voss-Weg 4 W-3550 Marburg (DE) Inventor: Dr. Patricia Oquendo Walter-Voss-Weg 4 W-3550 Marburg (DE Inventor: Dr. Gerd Zettlmeissl Am Hofacker 15 W-3551 Lahntal-Grossfelden (DE) Inventor: Dr. Brian Seed Fruit Street, Wellma Bldg. Boston, MA 02114 (DE) Representatives: Gerhard Aulmich et al., Hoechst AG Zentrale Patent Dept. P.O. Box 80 03 20 W-6230 Frankfurt am Main 80 (DE)

Fusion Proteins with Immunoglobulin Portions, Their Synthesis and Use

The invention relates to soluble fusion proteins, which are produced by genetic engineering and consist of human proteins or parts thereof, which do not belong to the immunoglobulin family, and of different portions of the constant region of immunoglobulin molecules. Surprisingly, the functional properties of the two fusion partners are retained in the fusion protein.

The invention relates to soluble fusion proteins, which are produced by genetic engineering and consist of human proteins or parts thereof, which do not belong to the immunoglobulin family, and of different portions of the constant region of immunoglobulin molecules. Surprisingly, the functional properties of the two fusion partners are retained in the fusion protein.

From the EP-A 0325 262 and the EP-A 0314 317, corresponding fusion proteins, consisting of different domains of the CD4-membrane protein of human T cells and of human IgG1 portions, are known. Some of these fusion proteins bind with the same affinity to the glycoprotein gp 120 of the human immunodeficiency virus as the cell-bound CD4 molecule. The CD4 molecule belongs to the immunoglobulin family and consequently, with respect to its tertiary structure, is built up very similarly to the immunoglobulin molecules. This is also true for the α chain of the T cell antigen receptor, for which such fusions have also been described (Gascoigne et al., Proc. Natl. Acad. Sci. USA, vol. 84 (1987), 2937 - 2940). In this case, the retention of the biological activity of the two fusion partners in the fusion protein was therefore to be expected on the basis of the very similar domain structure.

The human proteins, coupled pursuant to the invention preferably to the terminal amino group of the constant region of immunoglobulin, do not belong to the immunoglobulin family and are to be assigned to the following classes: (i) membrane-based proteins, the extracellular domains of which are brought into the fusion partially or totally. In particular, these are thromboplastin, cytokin receptors and growth factor receptors, such as the cellular receptors for interleukin-4, interleukin-7, tumor necrosis factor, GM-CSF, G-CSF, erythropoietin; (ii) soluble proteins, which are not membrane based and are brought partially or totally into the fusion. In particular, these are

proteins of therapeutic interest, such as erythropoietin and other cytokins and growth factors.

The fusion proteins can be produced in known prokaryotic and eukaryotic expression systems; preferably, however, they are produced in mammalian cells (such as CHO, COS and BHK cells).

Because of their immunoglobulin portions, the inventive fusion proteins can easily be purified by means of affinity chromatography and have improved *in vivo* pharmacokinetic properties.

In many cases, the Fc portion of the fusion protein is very advantageous for use in therapy and diagnosis and leads, for example, to improved pharmacokinetic properties (EP-A 0232 262). On the other hand, the possibility of removing the Fc portion, after the fusion protein was expressed, detected and purified in the described, advantageous manner, would be desirable for some applications. This is the case when the Fc portion proves to be an obstacle for the use in therapy and diagnosis, for example, when the fusion protein is to serve as antigen for immunizations.

There are various proteases, the use of which for this purpose appears to be conceivable. Papain or pepsin are used, for example, for producing F(ab) fragments from immunoglobulins (Immunology, published by I. Roitt et al., Gower Medical Publishing, London (1989)); however, they do not split particularly specifically. On the other hand, the blood-clotting factor Xa, recognizes the relatively infrequent tetrapeptide sequence Ile-Glu-Gly-Arg in a protein and carries out a hydrolytic splitting of the protein after the arginine group by splitting sequences, which contain the described tetrapeptide; they were introduced first by Nagai and Thogersen into a hybrid protein by genetic engineering (Nagai, K. and Thogersen, H.C., Nature, vol. 309 (1984), 810-812). These authors were able to show that the proteins,

expressed in E. coli, actually are split specifically by factor Xa. However, no example is known from the publications to show that such proteins can also be expressed in eukaryotic and particularly in animal cells and, after purification, be split by factor Xa. An expression of the inventive proteins in animal cells is, however, to be preferred since the expression of, for example, of normally membrane-bound receptors as fusion partners with retention of their native structure and, with that, of their biological activity is to be expected only in such a cell system. The expression into the supernatant of the cell culture facilitates the subsequent simple purification of the fusion protein.

The invention accordingly relates to soluble fusion proteins, which are produced by genetic engineering and consists of human proteins or parts thereof do not belong to the immunoglobulin family, and of different portions of the constant regions of heavy or light chains of immunoglobulins of different sub-classes (IgG, IgM, IgA, IgE). As immunoglobulin, the constant part of the heavy chain of human IgG is preferred and that of human IgG1 is especially preferred, the fusion taking place at the hinge region. In a special embodiment, the Fc portion can be severed simply by a splitting sequence, which is also incorporated and can be split by means of factor Xa.

The invention furthermore relates to methods for producing these fusion proteins by genetic engineering as well as to their use for diagnosis and therapy.

Finally, the invention is explained in further examples.

Example 1: Thromboplastin Fusion Proteins

Blood clotting is a process of central importance to the human organism. The clotting cascade, in which a plurality of cellular factors and plasma proteins interact, is regulated corresponding finely. The totality of these proteins (and their co-factors) is

referred to as clotting factors. End products of the clotting cascad are thrombin, which induces the aggregation of blood platelets, and fibrin, which stabilizes the platelet thrombus. Thrombin catalyses the formation of fibrin from fibrinogen and is itself formed from prothrombin by limited proteolysis. An activated factor X (factor Xa) is responsible for this step. In the presence of factor Va and calcium ions, it binds to the platelet membrane and splits prothrombin.

There are two ways for activating factor X, the extrinsic and the intrinsic pathway. In the intrinsic pathway, a series of factors is activated by proteolysis, in order to form self-active proteases. In the extrinsic pathway, thromboplastin (tissue factor) is synthesized increasingly by injured cells and activates factor X, together with factor VIIa and calcium ions. It was previously assumed that the activity of thromboplastin is limited to this reaction. However, the thromboplastin/VIIa complex also engages the intrinsic pathway in an activating manner at the level of factor IX. A thromboplastin/VIIa complex thus is one of the most important physiological activators of blood clotting.

It is therefore conceivable that thromboplastin, aside from its use as a diagnostic agent (see below), can also be used as a component of therapeutic agents for the treatment of inherited or acquired blood-clotting deficiencies. Examples of this are chronic hemophilias caused by a deficiency of factors VIII, IX or XI or also acute blood-clotting disorders as a result of, for example, liver of kidney diseases. The use of such therapeutic agents would also be conceivable after surgical interventions.

Thromboplastin is an integral membrane protein, which does not belong to the immunoglobulin family. Thromboplastin cDNA sequences have been published by a total of four groups (Fisher et al., Thromb. Res., vol. 48 (1987), 89-99; Morrisey et al., CelL, vol. 50 (1987), 129-135; Scarpati et al., Biochemistry, vol. 26 (1987), 5234-5238; Spicer et al., Proc. Natl. Acad. Sci. USA, vol. 84 (1987), 5148-5152). The

thromboplastin cDNA contains an open reading frame, which codes for a polypeptide of 295 amino acid groups, of which the 32 amino acids with a terminal nitrogen function as signal peptides. Mature thromboplastin consists of 263 amino acid groups and has a 3-domain structure: i) amino terminal, extracellular domains (219 amino acid groups); ii) transmembrane region (23 amino acid groups); iii) cytoplasmatic domains (carboxy-terminated; 21 amino acid groups). In the extracellular domains, three potential sites exist for N-glycosylation (Asn-X-Thr). Normally, thromboplastin is glycosylated; however, the glycosylation does not appear to be essential for the activity of the protein (Paborsky et al., Biochemistry, vol. 28 (1989), 8072-8077).

Thromboplastin is required as an addition to plasma samples in clotting diagnosis. The clotting status of the person being examined can be determined by the one-step prothrombin clotting time determination (such as the Quick test). The thromboplastin, required for diagnosis, is presently obtained from human tissue; the manufacturing process is difficult to standardize, the yield is low and considerable amounts of human starting material (placentas) must be made available. On the other hand, it is to be expected that the genetic engineering production of native, membrane-bound thromboplastin will also be problematical due to complex clotting methods. These problems can be avoided by the inventive fusion to immunoglobulin portions.

The inventive thromboplastin fusion proteins are expressed by mammalian cells (such as CHO, BHK and COS cells) into the culture medium, purified using affinity chromatography on protein A Sepharose and have surprisingly high activity in the one-step prothrombin clotting time determination.

Cloning Thromboplastin cDNA

The sequence, published by Scarpati et al., Biochemistry, vol. 26 (1987), 5234-5238, was used for cloning thromboplastin cDNA. Two ligonucleotid

probe molecules (see Figure 1) were derived from this. A cDNA bank from human placenta (Grundmann et al., Proc. Natl. Acad. Sci. USA, vol. 83 (1986), 8024-8028) was scanned with these two probe molecules.

cDNA clones of different lengths were obtained. One clone, 2b-Apr5, which is used for the further work, codes for the same amino acid sequence as the cDNA described in Scarpati et al. The total sequence of the 2b-Apr5 clone, together with the thromboplastin amino acid sequence derived therefrom, is shown in Figure 2.

Construction of a pTF1Fc Hybrid Plasmid Coating for Thromboplastin Fusion Protein

The pCD4E gamma 1 plasmid (EP 0 325 262 A2; deposited with the ATCC under the number 67610) serves for expressing a fusion protein from the human CD4 receptor and human IgG1. The DNA sequence, coding for the extracellular domains of CD4, is removed from this plasmid with the restriction enzyme HindIII and BamHI. Only a partial splitting may be carried out with the enzyme HindIII, in order to cut at only one of the two HindIII sites contained in pCD4E gamma 1 (position 2198). An opened vector is then present, for which a eukaryotic transcription regulation sequence (promoter) is followed by the open HindIII site. The open BamHI sites lies at the start of the coding regions for a pentapeptide linker, followed by the hinge and the CH2 and CH3 domains of the human IgG1. The reading frame in the BamHI recognition sequence GGATCC is such, that GAT is translated as aspartic acid. DNA amplification with heat-stable DNA polymerase makes it possible to change a bent sequence of DNA polymerase in such a manner, that random sequences are added at one or both ends. Two oligonucleotides were synthesized, which can hybridize with sequences in the 5' untranslated region (A: 5' GATCGATTAAGCTTCGGAACCCGCTCGATCTCG-CCGCC 3') or coding region(B: 5' GCATATCTGGATCCCCGTAGAATA-

TTTCTCTGAATTCCCC 3') of the thromboplastin cDNA. Oligonucleotide A moreover is partially homologous with the sequence of the coding strand, while oligonucleotide B is partially homologous with the non-coding strand (see Figure 3).

After the amplification, a DNA fragment (827 bp) is obtained, which contains a HindIII site at the 5' end (relative to the coding strand) before the start of the coding sequence and a BamHI site for the first three amino acid groups of the transmembrane region. The reading frame in the BamHI cutting site is such that, after ligation with the BamHI site in pCD4E gamma 1, a gene fusion is achieved with a continuous reading frame from the initiation codon of the thromboplastin cDNA up to the stop codon of the heavy chain of the IgG1. The desired fragment was obtained and, after treatment with HindIII and BamHI, ligated in the above-described vector pCD4E gamma 1, which has been cut with HindIII (partial)/BamHI. The resulting plasmid received the name of pTF1Fc Figure 4).

Transfection of pTF1Fc in Mammalian cells

The fusion protein, coded by the plasmid pTF1Fc, is referred to in the following as pTF1Fc. pTF1Fc was expressed transiently in COS cells. For this purpose, COS cells were transfected with the help of DEAE dextran (EP A 0325 262).

Indirect immunofluorescence investigations revealed that the proportion of transfected cells was about 25%. Twenty-four hours after transfection, the cells were transferred to a serum-free medium. This cell supernatant was harvested after a further three days.

Purification of pTF1Fc Fusion Protein from Cell Culture Supernatants

The supernatant (170 mL) of transiently transfected COS cells was collected over night in a batch process at 4°C with 0.8 mL of protein A Sepharose in a column, washed with 10 volumes of buffer (50 mM tris buffer, having a pH of 8.6 and containing 150 mM of NaCl) and eluted with elution buffer (100 mM of citric acid : 100 mM of sodium citrate 93 : 7) in 0.5 mL fractions. The first nine fractions were neutralized immediately and combined with 0.1 mL of 2 M tris buffer of pH 8.6 and the protein contained converted by three concentrating/diluting cycles in the Amicon Microconcentrator (Centricon 30) in TNE buffer (50 mM tris buffer having a pH of 7.4, 50 mM of NaCl, 1 mM EDTA). The pTF1Fc, so obtained, is electrophoretically pure in the SDS-PAGE (U.K. Lämmli, Nature 227 (1970) 680 - 685). In the absence of reducing agents, it behaves like a dimer (approximately 165 KDa) in the SDS-PAGE.

Biological Activity of Purified TF1Fc in the Prothrombin Clotting Time Determination

In low concentrations (> 50 ng/mL), TF1Fc fusion protein is active in the 1-step prothrombin clotting time determination (Vinazzer, H., Gerinnungs-physiologie und Methoden im Blutgerrinnungslabor (Clotting Physiology and Methods in the Blood Clotting Laboratory) (1979), published by Fisher, Stuttgart). The clotting times achieved are comparable with the clotting times, which are obtained with thromboplastin, which was isolated from human placenta.

Example 2: Interleukin-4 Receptor Fusion Proteins

Interleukin-4 (IL-4) is synthesized by T cells and was originally referred to as B cell growth factor, since it can stimulate the proliferation of B cells. It exerts a plurality of effects on these cells. In particular, these effects include the stimulation of the synthesis of molecules of the immunoglobulin classes IgG1 and IgE in activated B cells (Coffmann et al., Immunol. Rev., vol. 102 (1988) 5). Moreover, IL-4 also regulates the proliferation and differentiation of T cells and other hematopoietic cells. Accordingly, it contributes to the regulation of allergic and other immunological reactions. IL-4 binds to a specific receptor with high affinity. The cDNA, which codes for the human IL-4 receptor, was isolated (Idzerda et al., J. Exp. Med., vol. 171 (1990) 886 - 873). It is evident from the analysis of the amino acid sequence derived from the cDNA sequence that the IL-4 receptor consists of a total of 825 amino acids, the 25 N-terminal amino acids functioning as a signal peptide. Mature, human IL-4 receptor consists of 800 amino acids and, like thromboplastin, has a 3-domain structure: i) amino-terminal extracellular domains (207 amino acids); ii) transmembrane region (24 amino acids) and iii) cytoplasmatic domains (569 amino acids). In the extracellular domains, there are six potential sites for N-glycosidation (Asn-X-Thr/Ser). The IL-4 receptor has homologies with the human IL-6 receptor, with the β sub-unit of the human IL-2 receptor, with the mouse erythropoietin receptor and with the rat prolactin receptor (Idzerda et al., loc. cit). Accordingly, like thromboplastin, it is not a member of the immunoglobulin family; instead, together with the homologous proteins listed, it belongs to the new family of hematopoietin receptors. 4-Cysteine groups and a sequence (Trip-Ser-X-Trp-Ser) in the extracellular domain, located in the vicinity of the transmembrane region, jointly are members of this family.

A therapeutic use of a recombinant form of the IL-4 receptor to suppress the IL-4-induced immune reactions (such as transplant rejections, autoimmune diseases, allergic reactions), on the basis of the IL-4/IL-4 receptor system, of the function described, is possible.

The amount of substance required for therapy makes it necessary to produce such a substance by genetic engineering. Because of the problem-free purification by affinity chromatography and the improved pharmacokinetic properties, the synthesis of soluble forms of the IL-4 receptor as immunoglobulin fusion protein is particularly advantageous pursuant to the invention.

The IL-4 receptor fusion proteins are expressed by mammalian cells (such as CHO, BHK and COS cells) into the culture medium and purified by affinity chromatography on protein A Sepharose. Surprisingly, they have functional properties identical with those of the extracellular domains of the intact membrane-bound IL-4 receptor molecule.

Construction of a Hybrid Plasmid pIL-4RFc, Coding for IL-4 Receptor Fusion Proteins

If the plasmid pCD4E gamma l is cut with Xhol and BamHL, an opened vector is obtained, the open Xhol site of which is "downstream" from the promoter sequence. The open BamHI site lies at the start of the coding regions for a pentapeptide linker and is followed by the hinge domain and the CH2 and CH3 domains of human IgG1. The reading frame in the BamHI recognition sequence GGATCC is such that GAT is translated as aspartic acid. DNA amplification with heat-stable DNA polymerase enables a given sequence to be changed, so that any sequence can be added at one or both ends. Two oligonucleotides were synthesized, which can hybridize with sequences in the 5- untranslated region

(A: 5' GATCCAGTACTCGAGAGAGAAGCCGGGCGTGGTGGCTCATGC 3') or coding region

(B: 5, CTATGACATGGATCCTGCTCGAAGGGCTCCCTGTAGGAGTTGTG 3') of the IL-4 receptor cDNA, which is present in cloned form in the vector pDC302/T22-8 (Idzerda et al., loc. cit). Oligonucleotide A is partially homologous with the sequence of the coding strand, while the oligonucleotide B is partially homologous with the non-coding strand (see Figure 5). After amplification by means of the heat-stable DNA polymerase, a DNA fragment (836 bp) is obtained which, relative to the coding strand, contains an XhoI site at the 5' end before the start of the coding sequence and a BamHI site at the 3' end before the last codon of the extracellular domains. The reading frame in the BamHI cutting site is such that, after ligation with the BamHI site in pCD4E gamma 1, gene fusion is attained with a continuous reading frame from the initiation codon of the IL-4 receptor cDNA up to the stop codon of the heavy chain of the IgG1. The desired fragment was obtained and, after treatment with XhoI and BamHI, ligated in the above-described vector pCD4E, which is cut with XhoI/BamHI. The resulting plasmid received the name pIL4RFc (Figure 6).

Transfection of pIL4RFc in Mammalian cells

The fusion protein, coded by the plasmid pIL4RFc, is referred to in the following as pIL4RFc. pIL4RFc was expressed transiently in COS cells. For this purpose, COS cells were transfected with the help of DEAE dextran with pIL4RFc (EP A 0325 262). Indirect immunofluorescence analyses revealed that about 25% of the cells were transfected. About 24 hours after transfection, the cells were transferred to a serum-free medium. This cell supernatant was harvested after a further three days.

Purification of IL4RFc Fusion Protein From the Supernatant of the cell Culture

The supernatant (500 mL) of transiently transfected COS cells was collected overnight in a batch process at 4°C with 1.6 mL of protein A Sepharose in a column, washed with 10 volumes of washing buffer (50 mM of tris buffer, pH 8.8, 150 mM of NaCl) and eluted with elution buffer (100 mM citric acid : 100 mM sodium citrate 93 : 7) in 0.5 mL fractions. The first nine fractions were neutralized immediately in each case with 0.1 mL of 2M tris buffer of pH 8.8 and combined and the protein obtained converted by three concentrating/diluting cycles in the Amicon Microconcentrator (Centricon 30) in TNE buffer (50 mM tris buffer having a pH of 7.4, 50 mM of NaCl, 1 mM EDTA). The IL4RFc, so obtained, is electrophoretically pure in the SDS-PAGE (U.K. Lämmli, Nature 227 (1970) 680 - 685). In the absence of reducing agents, it behaves like a dimer (approximately 150 KDa) in the SDS-PAGE.

Biological Activity of Purified IL4RFc

IL4RFc protein binds radioactive ^{125}I-labeled IL4 with the same affinity (KD = 0.5 nM) as a membrane bound, intact IL-4 receptor. It inhibits the proliferation of the IL-4-dependent cell line CTILHuIL clone D (Idzerde et al., loc. cit) in concentrations of 10 - 1,000 ng/mL. Moreover, it is outstandingly suitable for the development of IL-4 binding tests, since it can be bound by its Fc part to microtiter plates precoated, for example, with rabbit antihuman IgG and in this form also binds its ligands with a high affinity.

Example 3: Erythropoietin Fusion Proteins

Mature erythropoietin (EPO) is a glycoprotein, which consists of 166 amino acids and is essential for the development of erythrocytes. It stimulates the maturation and the terminal differentiation of erythroid precursor cells. The cDNA for human EPO was cloned (EPA-0267 678) and coded for the 168 amino acids of the mature EPO and a signal peptide of 22 amino acids essential for the expression. With the help of cDNA, recombinant, functional EPO can be produced in mammalian cells modified by genetic engineering and used clinically for the treatment of anemic manifestations of different origins (for example, in the case of acute kidney failure).

Pursuant to the invention, the synthesis of EPO as an immunoglobulin fusion protein is particularly advantageous because of the problem-free purification and the improved pharmacokinetic properties.

Construction of a Hybrid Plasmid pEPOFc, Coding for Erythropoietin Fusion Proteins

This construction is effected similarly to that described in Example 2 (Section: "Construction of a Hybrid Plasmid pIL-4RFc, Coding for IL-4 Receptor Fusion Proteins"). Two oligonucleotides were synthesized, which can hybridize with sequences in the vicinity of the initiation codon
(A: 5'GATCGATCTCGAGATGGGGGTGCACGAATGTCCTGCCTGGCTGTGG 3') or the stop codon
(B: CTGGAATCGGATCCCCTGTCCTGCAGGCCTCCCCTGTGTACAGC 3') of the EPO cDNA cloned in the vector pCES (EP A 0267 678). Oligonucleotide A is partially homologous with the sequence of the coding strand and oligonucleotide B is partially homologous with the non-coding strand (see Figure 7). After the amplification by means of heat-stable DNA polymerase, a DNA fragment of 598 bp is

obtained which, relative to the coding strand, contains an XhoI site at the 5' end before the initiation code and in which the codon for the last but one C-terminal amino acid group of EPO (Asp) is present in a BamHI recognition sequence at the 3' end. The reading frame in the BamHI cutting site is such that, after ligation with the BamHI site in pCD4E gamma 1, a gene fusion is attained with a continuous reading frame from the initiation codon of the EPO cDNA up to the stop codon of the heavy chain of the IgG1. The desired fragment was obtained and ligated after treatment with XhoI and BamH1 in the above-described vector pCD4E gamma 1, which had been cut with XhoI/BamHI. The resulting plasmid was given the name of pEPOFc (Figure 6).

Claims

1. Soluble fusion proteins comprising human proteins, which do not belong to the immunoglobulin family, or parts thereof and different proportions of immunoglobulin molecules of all subclasses.

2. The fusion proteins of claim 1, characterized in that the immunoglobulin portion is the constant part of the heavy chain of human IgG.

3. The fusion proteins of claim 2, characterized in that the immunoglobulin portion is the constant part of the heavy chain of human IgG1 or a fragment thereof binding protein A.

4. The fusion proteins of claim 2 or claim 3, characterized in that the fusion takes place in the "hinge" region.

5. The fusion proteins of claims 1 to 4, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of a membrane protein or parts thereof.

6. The fusion proteins of claims 1 to 4, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of thromboplastin or parts thereof.

7. The fusion proteins of claims 1 to 4, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of a cytokin receptor or growth factor receptor or parts thereof.

8. The fusion proteins of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the IL-4 receptor or parts thereof.

9. The fusion proteins of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the IL-7 receptor or parts thereof.

10. The fusion proteins of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the tumor necrosis factor receptor or parts thereof.

11. The fusion proteins of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the G-CSF receptor or parts thereof.

12. The fusion proteins of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the GM-CSF receptor or parts thereof.

13. The fusion proteins of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the erythropoeitin receptor or parts thereof.

14. The fusion proteins of claims 1 to 4, characterized in that the protein, fused to immunoglobulin, is not a membrane-based, soluble protein or a part thereof.

15. The fusion proteins of claim 14, characterized in that the protein, fused to the immunoglobulin, is a cytokin receptor or a growth factor receptor or a part thereof.

16. The fusion proteins of claim 15, characterized in that the protein, fused to immunoglobulin, is erythropoietin or a part thereof.

17. The fusion proteins of claim 15, characterized in that the protein, fused to immunoglobulin, is GM-CSF or G-CSF or a part thereof.

18. The fusion protein of claim 15, characterized in that the protein, fused to immunoglobulin, is interleukin IL-1 to IL-8 or a part thereof.

19. The fusion proteins of one of the preceding claims 1 to 18, characterized in that additionally a factor Xa splitting site is inserted between the immunoglobulin part and the part, which is not an immunoglobulin.

20. A method for the preparation of fusion proteins of one of the claims 1 to 19, characterized in that the DNA, coding for these constructs, is introduced in a mammalian cell expression system and, after the expression, the fusion protein formed is purified over the immunoglobulin portion by means of affinity chromatography.

21. Use of the fusion proteins of one of the claims 1 to 19 for diagnosis.

22. Use of the fusion proteins of one of the claims 1 to 19 for therapy.

Claims f r the Following Convention C untry: ES

1. A method for the preparation of soluble fusion proteins comprising human proteins or parts thereof, which do not belong to the immunoglobulin family, and various portions of immunoglobulin molecules of all subclasses, characterized in that the DNA, coding for these constructs, is introduced into a prokaryotic or eukaryotic, preferably mammalian cell expression system and, after the expression, the fusion protein formed is purified over the immunoglobulin portion by means of affinity chromatography.

2. The method of claim 1, characterized in that the immunoglobulin portion is the constant part of the heavy chain of human IgG.

3. The method of claim 2, characterized in that the immunoglobulin portion is the constant part of the heavy chain of human IgG1 or a fragment thereof binding protein A.

4. The method of claim 2 or claim 3, characterized in that the fusion takes place in the "hinge" region.

5. The method of claims 1 to 4, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of a membrane protein or parts thereof.

6. The method of claims 1 to 4, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of thromboplastin or parts thereof.

7. The method of claims 1 to 4, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of a cytokin recept r or growth factor receptor or parts thereof.

8. The method of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the IL-4 receptor or parts thereof.

9. The method of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the IL-7 receptor or parts thereof.

10. The method of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the tumor necrosis factor receptor or parts thereof.

11. The method of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the G-CSF receptor or parts thereof.

12. The method of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the GM-CSF receptor or parts thereof.

13. The method of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the erythropoeitin receptor or parts thereof.

14. The method of claims 1 to 4, characterized in that the protein, fused to immunoglobulin, is not a membrane-based, soluble protein or a part thereof.

15. The method of claim 14, characterized in that the protein, fused to the immunoglobulin, is a cytokin receptor or a growth factor receptor or a part thereof.

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18. The fusion protein of claim 15, characterized in that the protein, fused to immunoglobulin, is interleukin IL-1 to IL-8 or a part thereof.

19. The method of one of the preceding claims 1 to 18, characterized in that additionally a factor Xa splitting site is inserted between the immunoglobulin part and the part, which is not an immunoglobulin.

Claims for the Following Convention Country: GR

1. Soluble fusion proteins comprising human proteins, which do not belong to the immunoglobulin family, or parts thereof and different proportions of immunoglobulin molecules of all subclasses.

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10. The fusion proteins of claim 7, characterized in that the protein, fused to the immunoglobulin, is the extracellular portion of the tumor necrosis factor receptor or parts thereof.

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18. The fusion protein of claim 15, characterized in that the protein, fused to immunoglobulin, is interleukin IL-1 to IL-8 or a part thereof.

19. The fusion proteins of one of the preceding claims 1 to 18, characterized in that additionally a factor Xa splitting site is inserted between the immunoglobulin part and the part, which is not an immunoglobulin.

20. A method for the preparation of fusion proteins of one of the claims 1 to 19, characterized in that the DNA, coding for these constructs, is introduced in a mammalian cell expression system and, after the expression, the fusion protein formed is purified over the immunoglobulin portion by means of affinity chromatography.

21. Use of the fusion proteins of one of the claims 1 to 19 for diagnosis.

Key for Figures:

Fortsetzung	continuation
Beginn	start
untranslatiert	not translated
Leserahmen	reading frame
Ende extrazelluläre Domäne	end of extracellular domains
Beginn Transmembranregion	start of the transmembrane region

European Patent Office

European Search Report

Application No.
EP 91110307.5

RELEVANT DOCUMENTS

Category	Characterization of the Documents, the Relevant Parts being given where Necessary	Relates to Claim	Classification of Application (Int. Cl⁵:)
Y	EP - A2 - 0 269 455 (TAKEDA CHEMICAL INDUSTRIES) * Abstract; Claims *	1 - 3, 20	C 07 K 15/06 C 12 N 15/62 A 61 K 37/02 A 61 K 39/395
D, Y	EP - A2 - 0 325 262 (THE GENERAL HOSPITAL Corporation * Claims 1, 3, 20, 23 *	1 - 3, 21	
P, A	EP - A2 - 0 414 178 THE GENERAL HOSPITAL Corporation * Claims 8, 12, 14, 20 *	1 - 3	**Fields Searched** C 07 K C 12 N A 61 K

This search report was prepared for all claims

Search Site	Final Date of Search	Examiner
Vienna	9-28-1991	Augustin

CATEGORY OF THE DOCUMENTS

X	of special importance considered alone	E	older patent document, which was published only now or after the filing date
Y	of special importance in conjunction with a different publication of the same category	D	document listed in the application
A	technological background	L	document listed for other reasons
O	nonwritten disclosure		
P	intermediate literature		
T	theory or fundamentals on which the invention is based	&	member of the same patent family, corresponding document